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U.S. APPLICATION NO. (If Known, see 37 CFR 1.5)

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INTERNATIONAL APPLICATION NO.
PCT/US00/17401

INTERNATIONAL FILING DATE
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25 June 1999

TITLE OF INVENTION
SOMATOSTATIN AGONISTS

APPLICANT(S) FOR DO/EO/US
Dean Sadat-Aalae and Barry A. Morgan

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. ☐ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern other documents or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
 - ☒ International Preliminary Examination Report
 - ☐
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09-980,943
PTO/PCT Rec'd 05 DEC 2001SOMATOSTATIN AGONISTSBackground of the Invention

5 The present invention is directed to cyclic peptides that have somatostatin
agonist activity, as defined by formula (I), shown and defined hereinbelow, or a
pharmaceutically acceptable salt thereof, pharmaceutical compositions comprising
said peptides and the use thereof as a somatostatin receptor subtypes agonist. The
peptides of the present invention bind selectively to the somatostatin subtype
10 receptor 5 and elicit an agonist effect from the somatostatin subtype receptors that
the peptides bind to.

Somatostatin (SRIF) is a cyclic tetradecapeptide hormone containing a
disulfide bridge between position 3 and position 14 (Heiman, et al.,
Neuroendocrinology, 45:429-436 (1987)) and has the properties of inhibiting the
release of growth hormone (GH) and thyroid-stimulating hormone (TSH), inhibiting
15 the release of amylin, insulin and glucagon, reducing gastric secretion and
neurotransmitter release. Metabolism of somatostatin by aminopeptidases and
carboxypeptidases leads to a short duration of action. Because of the short half-life
of the native somatostatin, various somatostatin analogs have been developed,
e.g., for the treatment of acromegaly. Raynor, et al., Molecular Pharmacol. 43:838
20 (1993).

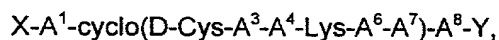
Five distinct somatostatin receptors have been identified and characterized.
Hoyer, et al., Naunyn-Schmiedeberg's Arch. Pharmacol., 350:441 (1994).
Somatostatin binds to five distinct receptor (SSTR) subtypes with relatively high and
equal affinity for each subtype. Binding to the different types of somatostatin
25 subtypes have been associated with the treatment of the following conditions and/or
diseases. ("SSTR-2") (Raynor, et al., Molecular Pharmacol. 43:838 (1993); Lloyd, et
al., Am. J. Physiol. 268:G102 (1995)) while the inhibition of insulin has been
attributed to the somatostatin type-5 receptor ("SSTR-5") (Coy, et al. 197:366-371
(1993)). Activation of types 2 and 5 have been associated with growth hormone
30 suppression and more particularly GH secreting adenomas (Acromegaly) and TSH
secreting adenomas. Activation of type 2 but not type 5 has been associated with
treating prolactin secreting adenomas. Other indications associated with activation

of the somatostatin subtypes are inhibition of insulin and/or glucagon and more particularly diabetes mellitus, angiopathy, proliferative retinopathy, dawn phenomenon and Nephropathy; inhibition of gastric acid secretion and more particularly peptic ulcers, enterocutaneous and pancreaticocutaneous fistula, irritable bowel syndrome, Dumping syndrome, watery diarrhea syndrome, AIDS related diarrhea, chemotherapy-induced diarrhea, acute or chronic pancreatitis and gastrointestinal hormone secreting tumors; treatment of cancer such as hepatoma; inhibition of angiogenesis, treatment of inflammatory disorders such as arthritis; retinopathy; chronic allograft rejection; angioplasty; preventing graft vessel and gastrointestinal bleeding. It is preferred to have an analog which is selective for the specific somatostatin receptor subtype responsible for the desired biological response, thus, reducing interaction with other receptor subtypes which could lead to undesirable side effects.

The peptides of formula (I) are a sub-genus encompassed by a genus of compounds described and claimed in copending U.S. Application No. 08/855,204, filed May 13, 1997, which application is assigned in part to the assignee of the present invention. The compounds of formula (I) of the present application are not specifically described in U.S. Application No. 08/855,204. It has been unexpectedly and surprisingly discovered that the compounds of formula (I) of the present invention possess somatostatin agonist activity. This is an unexpected and surprising discovery since the compounds of U.S. Application No. 08/855,204 were originally found to possess somatostatin antagonist activity.

Summary of the Invention

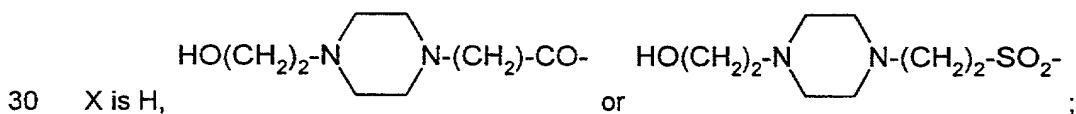
In one aspect, the present invention is directed to a peptide of the formula (I),



(I)

or a pharmaceutically acceptable salt thereof,

wherein



A¹ and A³ are each independently the D- or L-isomer of an amino acid selected from the group consisting of Phe, Tyr, Tyr(I), Trp, 3-Pal, 4-Pal, Cpa and Nal;

A⁴ is L-Trp, D-Trp, L-β-methyl-Trp or D-β-methyl-Trp;

A⁶ is -NH-(CHR¹)_n-CO-, where n is 2, 3, or 4;

5 A⁷ is L- or D-Cys;

A⁸ is the D- or L-isomer of an amino acid selected from the group consisting of Phe, Tyr, Tyr(I), Trp, Nal, Cpa, Val, Leu, Ile, Ser and Thr;

Y is NR²R³ where R² and R³ are each independently H or (C₁-C₅)alkyl;

R¹ is selected from the group consisting H, (C₁-C₄)alkyl and -CH₂-aryl; wherein said
10 aryl is an optionally substituted moiety selected from the group consisting of phenyl, 1-naphthyl, and 2-naphthyl, wherein said optionally substituted moiety is optionally substituted with one or more substituents each independently selected from the group consisting of (C₁₋₆)alkyl, (C₂₋₆)alkenyl, (C₂₋₆)alkynyl, aryl, aryl(C₁₋₆)alkyl, (C₁₋₆)alkoxy, -N(R⁴R⁵), -COOH, -CON(R⁴R⁵), halo, -OH, -CN, and -NO₂;

15 R⁴ and R⁵ each is, independently for each occurrence, H or (C₁₋₃)alkyl;
where the Cys of A² is bonded to the Cys of A⁷ by a di-sulfide bond formed from the thiol groups of each Cys.

A preferred group of peptides of the foregoing peptide of formula (I) is wherein

20 X is H;

A¹ is L-Phe, D-Phe, L-Cpa or D-Cpa;

A³ is L-Tyr, L-Trp or L-3-Pal;

A⁴ is D-Trp;

A⁶ is β-Ala or Gaba;

25 A⁷ is L-Cys;

A⁸ is Thr, L-Trp, L-Leu or L-Nal; and

R² and R³ are each H; or a pharmaceutically acceptable salt thereof.

Preferred peptides of the immediately foregoing group of peptides are:

Cpa-cyclo(D-Cys-3-Pal-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;

30 Cpa-cyclo(D-Cys-3-Pal-D-Trp-Lys-β-Ala-Cys)-Nal-NH₂;

Phe-cyclo(D-Cys-3-Pal-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;

Phe-cyclo(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;

Phe-cyclo(D-Cys-Trp-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;
Phe-cyclo(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Trp-NH₂;
D-Phe-cyclo(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;
D-Phe-cyclo(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Leu-NH₂; and

- 5 Phe-cyclo-(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Thr-NH₂; or a pharmaceutically acceptable salt thereof.

Preferred peptides of the immediately foregoing group of peptides are:

Cpa-cyclo(D-Cys-3-Pal-D-Trp-Lys-Gaba-Cys)-Nal-NH₂; and
Cpa-cyclo(D-Cys-3-Pal-D-Trp-Lys-β-Ala-Cys)-Nal-NH₂; or a pharmaceutically

- 10 acceptable salt thereof.

In another aspect, the present invention provides a pharmaceutical composition useful for eliciting a somatostatin agonist response in a human or other animal which comprises an effective amount of a peptide of formula (I) or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable

15 carrier.

In yet another aspect, the present invention provides a method of eliciting a somatostatin agonist response in a human or other animal in need thereof, which comprises administering an effective amount of a peptide of formula (I) or a pharmaceutically acceptable salt thereof to the human or other animal.

- 20 In a further aspect, the present invention provides a method of selectively binding a somatostatin subtype receptor type 5 in a human or other animal, which comprises administering an effective amount of a peptide of formula (I) or a pharmaceutically acceptable salt thereof to the human or other animal.

- In still a further aspect, the present invention provides a method of treating a
- 25 disease or condition in a human or other animal in need thereof, which comprises administering an effective amount of a peptide of formula (I) or a pharmaceutically acceptable salt thereof to the human or other animal, wherein said disease or condition is selected from the group consisting of Cushings Syndrome, gonadotropinoma, hyperparathyroidism, Paget's disease, VIPoma, nesidioblastosis,
- 30 hyperinsulinism, gastrinoma, Zollinger-Ellison Syndrome, hypersecretory diarrhea related to AIDS and other conditions, irritable bowel syndrome, pancreatitis, Crohn's Disease, systemic sclerosis, thyroid cancer, psoriasis, hypotension, panic

attacks, sclerodoma, small bowel obstruction, gastroesophageal reflux, duodenogastric reflux, Graves' Disease, polycystic ovary disease, upper gastrointestinal bleeding, pancreatic pseudocysts, pancreatic ascites, leukemia, meningioma, cancer cachexia, acromegaly, restenosis, hepatoma, lung cancer, melanoma, inhibiting the accelerated growth of a solid tumor, decreasing body weight, treating insulin resistance, Syndrome X, prolonging the survival of pancreatic cells, fibrosis, hyperlipidemia, hyperamylinemia, hyperprolactinemia and prolactinemia.

In still a further aspect, the present invention provides a method of inhibiting the secretion of growth hormone, insulin, glucagon or pancreatic exocrine secretion in a human or other animal in need thereof, which comprises administering a peptide of formula (I) or a pharmaceutically acceptable salt thereof to said human or other animal.

In an even further aspect, the present invention provides a method of imaging cells containing somatostatin receptors *in vivo* in a human or other animal, which comprises administering a peptide of formula (I), provided that at least one of A¹, A³ or A⁸ is Tyr(I), or a pharmaceutically acceptable salt thereof to said human or other animal.

In another aspect, the present invention provides a method of imaging cells containing somatostatin receptors *in vitro*, which comprises administering a peptide of formula (I), provided that at least one of A¹, A³ or A⁸ is Tyr(I), or a pharmaceutically acceptable salt thereof to said human or other animal. Such peptides of the present invention can be used either *in vivo* to detect cells having somatostatin receptors (e.g., cancer cells) or *in vitro* as a radioligand in a somatostatin receptor binding assay.

The three letter abbreviations accepted in the art are used to refer to the amino acids in a peptide of the present invention. In the formula set forth herein, the disulfide bond between the thiol group on the side chain of residue A₂ (i.e., D-Cys) and the thiol group on the side chain of residue A₇ (i.e., L-Cys or D-Cys) is not shown. The following amino acid abbreviations stand for the name indicated next to it: Cpa = p-chlorophenylalanine; Nal = β-(2-naphthyl)alanine; 3-Pal = β-(3-pyridyl)-alanine; 4-Pal = β-(4-pyridyl)-alanine; and Gaba = 4-aminobutyric acid. The

definition of "-NH-(CH₂)_n-CO- where n is 2, 3, or 4" encompasses such amino acids as β-Ala and Gaba.

Unless noted otherwise, the three letter abbreviation of an amino acid refers to the L-isomer.

5 The term alkyl is intended to include those alkyl groups of the designated length in either a straight or branched configuration. Exemplary of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tertiary butyl, pentyl, isopentyl, and the like. When the term C₀-alkyl is included in a definition it is intended to denote a single covalent bond.

10 The term alkenyl is intended to include hydrocarbon groups having one or more double bonds and the designated number of carbon atoms in either a straight or branched configuration. Exemplary of such alkenyl groups are ethenyl, propenyl, isopropenyl, butenyl, sec-butenyl, tertiary butenyl, pentenyl, isopentenyl, hexenyl, isohexenyl and the like.

15 The term alkynyl is intended to include those alkynyl groups, i.e., hydrocarbon groups having one or more triple bonds, having the designated number of carbon atoms in either a straight or branched configuration. Exemplary of such alkynyl groups are ethynyl, propynyl, butynyl, pentynyl, isopentynyl, hexynyl, isohexynyl and the like.

20 The term alkoxy is intended to include those alkoxy groups having the designated number of carbon atoms in either a straight or branched configuration. Exemplary of such alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, tertiary butoxy, pentoxy, isopentoxy, hexoxy, isohexoxy and the like.

25 The term aryl is intended to include aromatic rings known in the art, which can be mono-cyclic or bi-cyclic, such as phenyl and naphthyl.

The term halo is intended to include chlorine, bromine, iodine, and fluorine.

Detailed Description

30 One skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrations of the invention and is not meant to be construed as limiting the full scope of the invention.

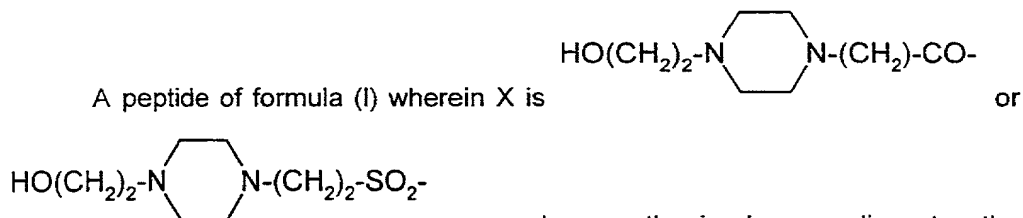
Peptides of the present invention can be and were synthesized on Rink Amide MBHA resin, (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucyl-MBHA resin), using a standard solid phase protocol for Fmoc chemistry and cleaved from the resin with a

5 TFA/Phenol/H₂O/triisopropylsilane (83ml/5g/10ml/2ml) mixture. Peptides were cyclized in CH₃CN/H₂O (5ml/5ml) using EKATHIOX™ resin (EKAGEN Corporation, San Carlos, CA) and purified on C₁₈ silica (Rainin Instruments Co., Woburn, MA now Varian Analytical, Walnut Creek, CA), using acetonitrile/0.1% trifluoroacetic acid buffers. Homogeneity was assessed by analytical HPLC and were

10 determined to be >95% for each peptide. Peptides were characterized by mass spectrometry.

The synthesis of iodinated Tyr (Tyr(I)) peptides of formula (I) of the present invention (e.g., the chloramine-T method) is well documented and are within the ability of a person of ordinary skill in the art. See, e.g., Czernick, et al., J. Biol.

15 Chem. 258:5525 (1993) and European Patent No. 389,180 B1.



, can be synthesized according to the processes and teachings of U.S. Patent No. 5,552,520, the contents of which are incorporated herein in its entirety.

20 Below is a detailed description of the synthesis of Examples 1 and 2. Other peptides within a compound of formula (I) can be prepared by making appropriate modifications, well-known to one of ordinary skill in the art of peptide synthesis.

EXAMPLE 1

Step 1 = Preparation of Fmoc-Cpa-S-trityl-D-Cys-Pal-N-in-t-Boc-D-Trp-N-ε-t-Boc-Lys-β-Ala-S-trityl-Cys-Nal-4-(2',4'-Dimethoxyphenylamino methyl) phenoxy-acetamido-norleucyl-4-methylbenzhydrylamine resin.

25

Rink amide MBHA resin (Novabiochem, Inc., San Diego, CA) 0.5g, (0.265 mmole), was placed in a reaction vessel of a 24-RV peptide synthesizer, assembled

by connecting a shaker (from the Burrell Wrist-Action Laboratory Shaker), a solvent distributor and a vacuum pump. The peptide synthesizer was programmed to perform the following reaction cycle:

- a. Dimethylformamide;
- 5 b. 25% piperidine in dimethylformamide (manually added) (2 times for
15 minutes each with 1 time wash with DMF in between);
- c. DMF washes (3 x 10 mL, 1 minute each);

The resin was stirred with Fmoc-Nal (1.06 mmol), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBUT) 1.007 mmole, and
10 diisopropylethyl amine (2.12 mmole) in dimethylformamide for about 1½ hours and the resulting amino acid resin was then cycled through steps (a) to (c) in the above washing/deblocking program.

The following amino acids were coupled successively to the Nal-resin by the
15 same procedure: Fmoc-S-Trityl-Cys, Fmoc-β-Ala, N-ε-t-Boc-Lys, Fmoc-(N-in-t-Boc)-D-Trp, Fmoc-Pal, Fmoc-S-trityl-D-Cys, and Fmoc-p-Cl-Phe.

After washing with DMF (3 x 10 mL, about 1 minute each) and drying under vacuum, the complete peptide resin weighed 0.749 g.

20 Step 2: Preparation of H-Cpa-cyclo(D-Cys-Pal-D-Trp-Lys-β-Ala-Cys)-Nal-NH₂

The peptide resin obtained from Step 1 of Example 1 (0.36 g, 0.087 mmole) was mixed with a freshly prepared solution of TFA (8.8 mL), phenol (0.5g), H₂O (0.5 mL) and triisopropylsilane (0.2 mL) at room temperature and stirred for about 2½
25 hours. Excess TFA was evaporated under reduced pressure to yield an oily residue. Ether was then added to the oily residue and the free linear peptide was precipitated, filtered, and washed with dry ether. The crude peptide was then dissolved in 10 mL of CH₃CN/H₂O (5 mL/5 mL), followed by the addition of 200 mg
30 EKATHIOX™ resin. The mixture was stirred overnight and filtered. The filtrate was evaporated to a small volume then applied to a column (22-250 mm) of microsorb octadecylsilane silica (5 μm). Elution with a linear gradient (20% to 40%, over 60 minutes) of acetonitrile in water, (both solvents have 0.1% trifluoroacetic acid) yields fractions which were examined by analytical high performance liquid

chromatography ("HPLC") and pooled to give maximum purity. Lyophilization of the solution from water gave 26 mg of the product as white, fluffy powder. The product was found to be homogeneous by HPLC C₁₈ silica using the same eluant as described above and a linear gradient (30% to 70%, over 15 min) (Retention Time - 5 6.313 minutes). Infusion mass spectrometry confirmed the composition of the cyclic octapeptide, MW 1133.8.

EXAMPLE 2

Step 1: Preparation of Fmoc-Cpa-S-trityl-D-Cys-Pal-in-t-Boc-D-Trp-N-ε-t-Boc-Lys-Gaba-S-trityl-Cys-Nal-4-(2',4'-Dimethoxyphenylaminomethyl)
10 Phenoxyacetamido-norleucyl-4-methylbenzhydrylamine resin

Rink amide MBHA resin (Novabiochem, Inc. San Diego, CA) 0.2 g, (0.106 mmole) was placed in reaction vessel #3, (RV-3) of the 24-RV peptide synthesizer. The peptide synthesizer was programmed to perform the following reaction cycle:

- a. Dimethylformamide;
- 15 b. 25% piperidine in dimethylformamide (manually added) (2 times for 15

minutes each with 1 time wash with DMF in between);

- c. DMF washes (3 x 10 mL, 1 minute each);

The resin was stirred with Fmoc-Nal (0.424 mmol), 2-(1H-benzotriazol-1-yl)-
20 1,1,3,3-tetramethyluronium hexafluorophosphate (HBUT) 0.403 mmole), and diisopropylethyl amino (0.848 mmole) in dimethylformamide for about 1½ hours and the resulting amino acid resin was then cycled through steps (a) to (c) in the above wash program.

The following amino acids were coupled successively to the peptide resin by
25 the same procedure: Fmoc-S-Trityl-Cys, Fmoc-Gaba, N-ε-t-Boc-Lys, Fmoc-(N-in-t-Boc)-D-Trp, Fmoc-Pal, Fmoc-S-trityl-D-Cys, and Fmoc-Cpa. After washing with DMF (3 x 10 mL, about 1 minute each) and drying under vacuum, the complete resin weighed 0.31 g.

Step 2: Preparation of H-Cpa-cyclo(D-Cys-Pal-D-Trp-Lys-Gaba-Cys)-Nal-NH₂

30 The peptide resin obtained from Step 1 of Example 2 was mixed with a freshly prepared solution of TFA (8.3 mL), phenol (0.5g), H₂O (1 mL) and triisopropylsilane (0.2 mL) at room temperature and stirred for about 2½ hours.

Excess TFA was evaporated under reduced pressure to give an oily residue. Ether was then added to the oily residue and the free linear peptide was precipitated, filtered, and then washed with dry ether. The crude peptide was then dissolved in 10 mL of CH₃CN/H₂O followed by the addition of 200 mg of EKATHIOX™ resin.

5 The mixture was stirred overnight and filtered. The filtrate was evaporated to a small volume then applied to a column (22-250 mm) of microsorb octadecylsilane silica (5 µm), and eluted with a linear gradient (20 % to 100 %, over 60 minutes) of acetonitrile in water, in which both solvents have 0.1% trifluoroacetic acid. Fractions were examined by analytical high performance liquid chromatography (HPLC) and pooled to give maximum purity. Lyophilization of the solutions from water gave 13 mg of the product as white, fluffy powder. The product was found to be homogeneous by HPLC C₁₈ silica using the same eluant as described above (20% to 80%, over 15 min) (Retention time - 9.195 minutes). Infusion mass spectrometry confirmed the composition of the cyclic octapeptide, MW 1147.83.

15 The peptides of this invention can be provided in the form of pharmaceutically acceptable salts. Examples of such salts include, but are not limited to, those formed with organic acids (e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, methanesulfonic, toluenesulfonic, or pantoic acid), inorganic acids (e.g., hydrochloric acid, sulfuric acid, or phosphoric acid), and polymeric acids (e.g., tannic acid, carboxymethyl cellulose, polylactic, polyglycolic, or copolymers of polylactic-glycolic acids). A typical method of making a salt of a peptide of the present invention is well known in the art and can be accomplished by standard methods of salt exchange. Accordingly, the TFA salt of a peptide of the present invention (the TFA salt results from the purification of the peptide by using preparative HPLC, eluting with TFA containing buffer solutions) can be converted into another salt, such as an acetate salt by dissolving the peptide in a small amount of 0.25 N acetic acid aqueous solution. The resulting solution is applied to a semi-prep HPLC column (Zorbax, 300 SB, C-8). The column is eluted with (1) 0.1N ammonium acetate aqueous solution for 0.5 hrs., (2) 0.25N acetic acid aqueous solution for 0.5 hrs. and (3) a linear gradient (20% to 100% of solution B over 30 min.) at a flow rate of 4 ml/min (solution A is 0.25N acetic acid aqueous solution; solution B is 0.25N acetic acid in acetonitrile/water, 80:20). The fractions containing

the peptide are collected and lyophilized to dryness.

The affinity of a peptide of the present invention for human somatostatin subtype receptors 1 to 5 (ss_{t1} , ss_{t2} , ss_{t3} , ss_{t4} and ss_{t5} , respectively) is determined by measuring the inhibition of (125 I-Tyr 11)SRIF-14 binding to CHO-K1 transfected cells.

5 The human ss_{t1} receptor gene was cloned as a genomic fragment. A 1.5 Kb *Pst*I-*Xmn*I segment containing 100 bp of the 5'-untranslated region, 1.17 Kb of the entire coding region, and 230 bp of the 3'-untranslated region was modified by the *Bgl*II linker addition. The resulting DNA fragment was subcloned into the *Bam*HI site of a pCMV-81 to produce the mammalian expression plasmid (provided by Dr. Graeme Bell, Univ. Chicago). A clonal cell line stably expressing the ss_{t1} receptor was obtained by transfection into CHO-K1 cells (ATCC) using the calcium phosphate co-precipitation method (1). The plasmid pRSV-neo (ATCC) was included as a selectable marker. Clonal cell lines were selected in RPMI 1640 media containing 0.5 mg/ml of G418 (Gibco), ring cloned, and expanded into culture.

15 The human ss_{t2} somatostatin receptor gene, isolated as a 1.7Kb *Bam*HI-*Hind*III genomic DNA fragment and subcloned into the plasmid vector pGEM3Z (Promega), was kindly provided by Dr. G. Bell (Univ. of Chicago). The mammalian cell expression vector is constructed by inserting the 1.7Kb *Bam*HI-*Hind*III fragment into compatible restriction endonuclease sites in the plasmid pCMV5. A clonal cell line is obtained by transfection into CHO-K1 cells using the calcium phosphate co-precipitation method. The plasmid pRSV-neo is included as a selectable marker.

20 The human ss_{t3} was isolated as a genomic fragment, and the complete coding sequence was contained within a 2.4 Kb *Bam*HI/*Hind*III fragment. The mammalian expression plasmid, pCMV-h3 was constructed by inserting the a 2.0 Kb *Nco*I-*Hind*III fragment into the *Eco*R1 site of the pCMV vector after modification of the ends and addition of *Eco*R1 linkers. A clonal cell line stably expressing the ss_{t3} receptor was obtained by transfection into CHO-K1 cells (ATCC) using the calcium phosphate co-precipitation method. The plasmid pRSV-neo (ATCC) was included as a selectable marker. Clonal cell lines were selected in RPMI 1640 media containing 0.5 mg/ml of G418 (Gibco), ring cloned, and expanded into culture.

The human *sst*₄ receptor expression plasmid, pCMV-HX was provided by Dr. Graeme Bell (Univ. Chicago). The vector contains the 1.4 Kb *NheI-NheI* genomic fragment encoding the human *sst*₄, 456 bp of the 5'-untranslated region and 200 bp of the 3'-untranslated region, clone into the *XbaI/EcoR1* sites of PCMV-HX. A clonal cell line stably expressing the *sst*₄ receptor was obtained by transfection into CHO-K1 cells (ATCC) using the calcium phosphate co-precipitation method. The plasmid pRSV-neo (ATCC) was included as a selectable marker. Clonal cell lines were selected in RPMI 1640 media containing 0.5 mg/ml of G418 (Gibco), ring cloned, and expanded into culture.

The human *sst*₅ gene was obtained by PCR using a λ genomic clone as a template, and kindly provided by Dr. Graeme Bell (Univ. Chicago). The resulting 1.2 Kb PCR fragment contained 21 base pairs of the 5'-untranslated region, the full coding region, and 55 bp of the 3'-untranslated region. The clone was inserted into *EcoR1* site of the plasmid pBSSK(+). The insert was recovered as a 1.2 Kb *HindIII-XbaI* fragment for subcloning into pCVM5 mammalian expression vector. A clonal cell line stably expressing the *SST*₅ receptor was obtained by transfection into CHO-K1 cells (ATCC) using the calcium phosphate co-precipitation method. The plasmid pRSV-neo (ATCC) was included as a selectable marker. Clonal cell lines were selected in RPMI 1640 media containing 0.5 mg/ml of G418 (Gibco), ring cloned, and expanded into culture.

CHO-K1 cells stably expressing one of the human *sst* receptor are grown in RPMI 1640 containing 10% fetal calf serum and 0.4 mg/ml geneticin. Cells are collected with 0.5 mM EDTA, and centrifuged at 500 g for about 5 min. at about 4°C. The pellet is resuspended in 50 mM Tris, pH 7.4 and centrifuged twice at 500 g for about 5 min. at about 4°C. The cells are lysed by sonication and centrifuged at 39000 g for about 10 min. at about 4°C. The pellet is resuspended in the same buffer and centrifuged at 50000 g for about 10 min. at about 4°C and membranes in resulting pellet are stored at - 80°C.

Competitive inhibition experiments of (¹²⁵I-Tyr¹¹)SRIF-14 binding are run in duplicate in polypropylene 96 well plates. Cell membranes (10 μ g protein/well) are incubated with (¹²⁵I-Tyr¹¹)SRIF-14 (0.05 nM) for about 60 min. at about 37°C in 50 mM

HEPES (pH 7.4), 0.2% BSA, 5 mM MgCl₂, 200 KIU/ml Trasylol, 0.02 mg/ml bacitracin and 0.02 mg/ml phenylmethanesulphonyl fluoride.

Bound from free (¹²⁵I-Tyr¹¹)SRIF-14 is separated by immediate filtration through GF/C glass fiber filter plate (Unifilter, Packard) presoaked with 0.1 % polyethylenimine (P.E.I.), using Filtermate 196 (Packard) cell harvester. Filters are washed with 50 mM HEPES at about 0-4°C for about 4 sec. and assayed for radioactivity using Packard Top Count.

Specific binding is obtained by subtracting nonspecific binding (determined in the presence of 0.1 μM SRIF-14) from total binding. Binding data are analyzed by computer-assisted nonlinear regression analysis (MDL) and inhibition constant (K_i) values are determined.

The determination of whether a compound of the instant invention is an agonist or an antagonist is determined by the following assay.

Functional assay: Inhibition of cAMP intracellular production:

CHO-K1 Cells expressing human somatostatin (SRIF-14) subtype receptors are seeded in 24-well tissue culture multidishes in RPMI 1640 media with 10% FCS and 0.4 mg/ml geneticin. The medium is changed the day before the experiment.

Cells at 10⁵ cells/well are washed 2 times by 0.5 ml and fresh RPMI with 0.2% BSA supplemented with 0.5 mM (1) 3-isobutyl-1-methylxanthine (IBMX) and incubated for about 5 min at about 37°C.

- Cyclic AMP production is stimulated by the addition of 1mM forskolin (FSK) for about 15-30 minutes at about 37°C.
- The agonist effect of a compound is measured by the simultaneous addition of FSK (1μM), SRIF-14 (10⁻¹² M to 10⁻⁶ M) and a test compound (10⁻¹⁰ M to 10⁻⁵ M).
- The antagonist effect of a compound is measured by the simultaneous addition of FSK (1μM), SRIF-14 (1 to 10 nM) and a test compound (10⁻¹⁰ M to 10⁻⁵ M).

The reaction medium is removed and 200 μl 0.1 N HCl is added. cAMP is measured using radioimmunoassay method (Kit FlashPlate SMP001A, New England Nuclear).

As is well known to those skilled in the art, the known and potential uses of somatostatin are varied and multitudinous. Thus, the administration of a peptide of

this invention for purposes of stimulating the somatostatin receptors can have the same effects or uses as somatostatin itself. For example, inhibiting the secretion of growth hormone, insulin, glucagon and pancreatic exocrine secretion (U.S. Patent No. 4,853,371); for treating restenosis (U.S. Patent No. 5,147,856); for treating
5 hepatoma (U.S. Patent No. 5,411,943); for treating lung cancer (U.S. Patent No. 5,073,541); treating melanoma (U.S. Application No. 08/089,410 filed July 9, 1993); for inhibiting the accelerated growth of a solid tumor (U.S. Patent No. 5,504,069); for decreasing body weight (U.S. Application No. 08/854,941 filed May 13, 1997); for treating insulin resistance and Syndrome X (U.S. Application No. 08/854,943
10 filed May 13, 1997); for prolonging the survival of pancreatic cells (U.S. Patent No. 5,688,418); for treating fibrosis (PCT Application No. PCT/US97/14154); for treating hyperlipidemia (U.S. Application No. 08/855,311 filed May 13, 1997); for treating hyperamylinemia (U.S. Application No. 08/440,061 filed May 12, 1995); for treating hyperprolactinemia and prolactinomas (U.S. Application No. 08/852,221 filed May
15 7, 1997); Cushings Syndrome (see Clark, R.V. et al, Clin. Res. 38, p. 943A, 1990); gonadotropinoma (see Ambrosi B., et al., Acta Endocr. (Copenh.) 122, 569-576, 1990); hyperparathyroidism (see Miller, D., et al., Canad. Med. Ass. J., Vol. 145, pp. 227-228, 1991); Paget's disease (see, Palmieri, G.M.A., et al., J. of Bone and Mineral Research, 7, (Suppl. 1), p. S240 (Abs. 591), 1992); VIPoma (see
20 Koberstein, B., et al., Z. Gastroenterology, 28, 295-301, 1990 and Christensen, C., Acta Chir. Scand. 155, 541-543, 1989); nesidioblastosis and hyperinsulinism (see Laron, Z., Israel J. Med. Sci., 26, No. 1, 1-2, 1990, Wilson, D.C., Irish J. Med. Sci., 158, No. 1, 31-32, 1989 and Micic, D., et al., Digestion, 16, Suppl. 1.70. Abs. 193, 1990); gastrinoma (see Bauer, F.E., et al., Europ. J. Pharmacol., 183, 55 1990);
25 Zollinger-Ellison Syndrome (see Mozell, E., et al., Surg. Gynec. Obstet., 170, 476-484, 1990); hypersecretory diarrhea related to AIDS and other conditions (due to AIDS, see Cello, J.P., et al., Gastroenterology, 98, No. 5, Part 2, Suppl., A163 1990; due to elevated gastrin-releasing peptide, see Alhindawi, R., et al., Can. J. Surg., 33, 139-142, 1990; secondary to intestinal graft vs. host disease, see Bianco
30 J.A., et al., Transplantation, 49, 1194-1195, 1990; diarrhea associated with chemotherapy, see Petrelli, N., et al., Proc. Amer. Soc. Clin. Oncol., Vol. 10, P 138, Abstr. No. 417 1991); irritable bowel syndrome (see O'Donnell, L.J.D., et al.,

Aliment. Pharmacol. Therap., Vol. 4., 177-181, 1990); pancreatitis (see Tulassay, Z., et al., Gastroenterology, 98, No. 5, Part 2, Suppl., A238, 1990); Crohn's Disease (see Fedorak, R.N., et al., Can. J. Gastroenterology, 3, No. 2, 53-57, 1989); systemic sclerosis (see Soudah, H., et al., Gastroenterology, 98, No. 5, Part 2, 5 Suppl., A129, 1990); thyroid cancer (see Modigliani, E., et al., Ann., Endocr. (Paris), 50, 483-488, 1989); psoriasis (see Camisa, C., et al., Cleveland Clinic J. Med., 57, No. 1, 71-76, 1990); hypotension (see Hoeldtke, R.D., et al., Arch. Phys. Med. Rehabil., 69, 895-898, 1988 and Kooner, J.S., et al., Brit. J. Clin. Pharmacol., 28, 735P-736P, 1989); panic attacks (see Abelson, J.L., et al., Clin. Psychopharmacol., 10, 128-132, 1990); scleroderma (see Soudah, H., et al., Clin. Res., Vol. 39, p. 303A, 1991); small bowel obstruction (see Nott, D.M., et al., Brit. J. Surg., Vol. 77, p. A691, 1990); gastroesophageal reflux (see Branch, M.S., et al., Gastroenterology, Vol. 100, No. 5, Part 2 Suppl., p. A425, 1991); duodenogastric reflux (see Hasler, W., et al., Gastroenterology, Vol. 100, No. 5, Part 2, Suppl., p. A448, 1991); Graves' Disease (see Chang, T.C., et al., Brit. Med. J., 304, p. 158, 1992); polycystic ovary disease (see Prelevic, G.M., et al., Metabolism Clinical and Experimental, 41, Suppl. 2, pp 76-79, 1992); upper gastrointestinal bleeding (see Jenkins, S.A., et al., Gut., 33, pp. 404-407, 1992 and Arrigoni, A., et al., American Journal of Gastroenterology, 87, p. 1311, (abs. 275), 1992); pancreatic pseudocysts and ascites (see Hartley, J.E., et al., J. Roy. Soc. Med., 85, pp. 107-108, 1992); leukemia (see Santini, et al., 78, (Suppl. 1), p. 429A (Abs. 1708), 1991); meningioma (see Koper, J.W., et al., J. Clin. Endocr. Metab., 74, pp. 543-547, 1992); and cancer cachexia (see Bartlett, D.L., et al., Surg. Forum., 42, pp. 14-16, 1991).

25 Accordingly, the present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, at least one of the peptides of formula (I) in association with a pharmaceutically acceptable carrier.

In general an effective dosage for the activities of this invention, for example the treatment of acromegaly, is in the range of 0.01 to 200 mg/kg/day, preferably 0.5 30 to 100 mg/kg/day.

A peptide of this invention can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous or subcutaneous injection, or implant),

nasal, vaginal, rectal, sublingual or topical routes of administration and can be formulated with pharmaceutically acceptable carriers to provide dosage forms appropriate for each route of administration.

Solid dosage forms for oral administration include capsules, tablets, pills, 5 powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than such inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the 10 dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, 15 compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring and perfuming agents.

Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, 20 vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the 25 compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients 30 such as coca butter or a suppository wax.

Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

Further, a compound of this invention can be administered in a sustained release composition such as those described in the following patents and patent applications. U.S. Patent No. 5,672,659 teaches sustained release compositions comprising a bioactive agent and a polyester. U.S. Patent No. 5,595,760 teaches sustained release compositions comprising a bioactive agent in a gelable form. U.S. Patent No. 5,821,221, teaches polymeric sustained release compositions comprising a bioactive agent and chitosan. U.S. Application No. 08/740,778 filed November 1, 1996, teaches sustained release compositions comprising a bioactive agent and cyclodextrin. U.S. Application No. 09/015,394 filed January 29, 1998, teaches absorbable sustained release compositions of a bioactive agent. U.S. Application No. 09/121,653 filed July 23, 1998, teaches a process for making microparticles comprising a therapeutic agent such as a peptide in an oil-in-water process. U.S. Application No. 09/131,472 filed August 10, 1998, teaches complexes comprising a therapeutic agent such as a peptide and a phosphorylated polymer. U.S. Application No. 09/184,413 filed November 2, 1998, teaches complexes comprising a therapeutic agent such as a peptide and a polymer bearing a non-polymerizable lactone. The teachings of the foregoing patents and applications are incorporated herein by reference.

The dosage of active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient be such that a suitable dosage form is obtained. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment. Generally, dosage levels of between 0.0001 to 100 mg/kg of body weight daily are administered to humans and other animals, e.g., mammals, to obtain effective release of growth hormone.

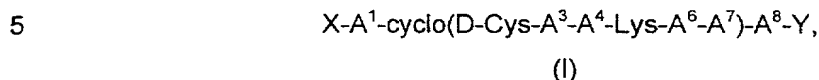
A preferred dosage range is 0.01 to 5.0 mg/kg of body weight daily which can be administered as a single dose or divided into multiple doses.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference.

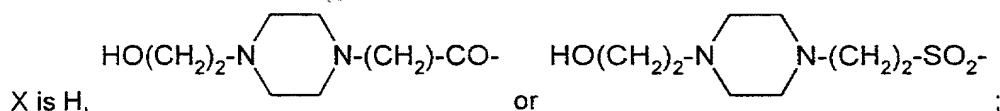
CLAIMS

What is claimed is:

1. A peptide of the formula (I),



or a pharmaceutically acceptable salt thereof ,
wherein



10 A^1 and A^3 are each independently the D- or L-isomer of an amino acid selected from the group consisting of Phe, Tyr, Tyr(I), Trp, 3-Pal, 4-Pal, Cpa and Nal;

A^4 is L-Trp, D-Trp, L- β -methyl-Trp or D- β -methyl-Trp;

A^6 is $\text{-NH-(CHR}^1\text{)}_n\text{-CO-}$, where n is 2, 3, or 4;

A^7 is L- or D-Cys;

15 A^8 is the D- or L-isomer of an amino acid selected from the group consisting of Phe, Tyr, Tyr(I), Trp, Nal, Cpa, Val, Leu, Ile, Ser and Thr;

Y is NR^2R^3 where R^2 and R^3 are each independently H or $(C_1\text{-C}_5)\text{alkyl}$;

R^1 is selected from the group consisting H, $(C_1\text{-C}_4)\text{alkyl}$ and $\text{-CH}_2\text{-aryl}$; wherein said aryl is an optionally substituted moiety selected from the group consisting of phenyl,

20 1-naphthyl, and 2-naphthyl, wherein said optionally substituted moiety is optionally substituted with one or more substituents each independently selected from the group consisting of $(C_{1-6})\text{alkyl}$, $(C_{2-6})\text{alkenyl}$, $(C_{2-6})\text{alkynyl}$, aryl, aryl $(C_{1-6})\text{alkyl}$, $(C_{1-6})\text{alkoxy}$, $\text{-N(R}^4\text{R}^5\text{)}$, -COOH , $\text{-CON(R}^4\text{R}^5\text{)}$, halo, -OH , -CN , and -NO_2 ;

R^4 and R^5 each is, independently for each occurrence, H or $(C_{1-3})\text{alkyl}$;

25 where the Cys of A^2 is bonded to the Cys of A^7 by a di-sulfide bond formed from the thiol groups of each Cys.

2. A peptide according to claim 1 wherein

A^1 is L-Phe, D-Phe, L-Cpa or D-Cpa;

A^3 is L-Tyr, L-Trp or L-3-Pal;

30 A^4 is D-Trp;

A⁶ is β -Ala or Gaba;

A⁷ is L-Cys;

A⁸ is L-Thr, L-Trp, L-Leu or L-Nal; and

R² and R³ are each H;

5 or a pharmaceutically acceptable salt thereof.

3. A peptide according to claim 2 wherein said peptide is of the formula

Cpa-cyclo(D-Cys-3-Pal-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;

Cpa-cyclo(D-Cys-3-Pal-D-Trp-Lys- β -Ala-Cys)-Nal-NH₂;

Phe-cyclo(D-Cys-3-Pal-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;

10 Phe-cyclo(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;

Phe-cyclo(D-Cys-Trp-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;

Phe-cyclo(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Trp-NH₂;

D-Phe-cyclo(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Nal-NH₂;

D-Phe-cyclo(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Leu-NH₂; or

15 Phe-cyclo(D-Cys-Tyr-D-Trp-Lys-Gaba-Cys)-Thr-NH₂;

or a pharmaceutically acceptable salt thereof.

4. A peptide according to claim 3 wherein said peptide is of the formula

Cpa-cyclo(D-Cys-3-Pal-D-Trp-Lys-Gaba-Cys)-Nal-NH₂; or

Cpa-cyclo(D-Cys-3-Pal-D-Trp-Lys- β -Ala-Cys)-Nal-NH₂;

20 or a pharmaceutically acceptable salt thereof.

5. A pharmaceutical composition useful for eliciting a somatostatin agonist response in a human or other animal which comprises an effective amount of a peptide of formula (I) according to claim 1 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

25 6. A method of eliciting a somatostatin agonist response in a human or other animal in need thereof, which comprises administering an effective amount of a peptide of formula (I) according to claim 1 or a pharmaceutically acceptable salt thereof to the human or other animal.

30 7. A method of selectively binding a somatostatin subtype receptor type 5 in a human or other animal, which comprises administering an effective amount of a peptide of formula (I) according to claim 1 or a pharmaceutically acceptable salt thereof to the human or other animal.

8. A method of treating a disease or condition in a human or other animal in need thereof, which comprises administering a peptide of formula (I) or a pharmaceutically acceptable salt thereof to said human or other animal, wherein said disease or condition is selected from the group consisting of Cushing's Syndrome, gonadotropinoma, hyperparathyroidism, Paget's disease, VIPoma, nesidioblastosis, hyperinsulinism, gastrinoma, Zollinger-Ellison Syndrome, hypersecretory diarrhea related to AIDS and other conditions, irritable bowel syndrome, pancreatitis, Crohn's Disease, systemic sclerosis, thyroid cancer, psoriasis, hypotension, panic attacks, scleroderma, small bowel obstruction, gastroesophageal reflux, duodenogastric reflux, Graves' Disease, polycystic ovary disease, upper gastrointestinal bleeding, pancreatic pseudocysts, pancreatic ascites, leukemia, meningioma, cancer cachexia, acromegaly, restenosis, hepatoma, lung cancer, melanoma, inhibiting the accelerated growth of a solid tumor, decreasing body weight, treating insulin resistance, Syndrome X, prolonging the survival of pancreatic cells, fibrosis, hyperlipidemia, hyperamylinemia, hyperprolactinemia and prolactinemia.

9. A method of inhibiting the secretion of growth hormone, insulin, glucagon or pancreatic exocrine secretion in a human or other animal in need thereof, which comprises administering a peptide of formula (I) according to claim 1 or a pharmaceutically acceptable salt thereof to said human or other animal.

10. A method of imaging cells containing somatostatin receptors *in vivo* in a human or other animal, which comprises administering a peptide of formula (I) according to claim 1, provided that at least one of A¹, A³ or A⁸ is Tyr(I), or a pharmaceutically acceptable salt thereof to said human or other animal.

11. A method of imaging cells containing somatostatin receptors *in vitro*, which comprises administering a peptide of formula (I) according to claim 1, provided that at least one of A¹, A³ or A⁸ is Tyr(I), or a pharmaceutically acceptable salt thereof to said human or other animal.

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled SOMATOSTATIN AGONISTS, the specification of which:

☐ is attached hereto.

☒ was filed on December 5, 2001 as Application Serial No. 09/980,943 and was amended on _____.

☒ was described and claimed in PCT International Application No. PCT/US00/17401 filed on June 23, 2000 and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

<u>U.S. Serial No.</u>	<u>Filing Date</u>	<u>Status</u>
60/141,028	06/25/1999	Expired

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

<u>U.S. Serial No.</u>	<u>Filing Date</u>	<u>Status</u>
------------------------	--------------------	---------------

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

<u>Country</u>	<u>Application No.</u>	<u>Filing Date</u>	<u>Priority Claimed</u>
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

Combined Declaration and Power of Attorney
Page 2 of 2 Pages

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Y. Rocky Tsao, Reg. No. 34,053; Brian R. Morrill Reg. No. 42,098; Alan F. Feeney Reg. No. 43,609; Timothy A. French, Reg. No. 30,175; Eric L. Prah, Reg. No. 32,590; Jeffrey D. Hsi, Reg. No. 40,024; and Frank R. Occhuiti, Reg. No. 35,306.

Address all telephone calls to Brian R. Morrill at telephone number (508) 478-0144.

Address all correspondence to Brian R. Morrill, Esq. at:

Biomeasure, Incorporated
27 Maple Street
Milford, MA 01757

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name of Inventor: DEAN SADAT-AALAE

Inventor's Signature: _____ Date: _____
Residence Address: La Jolla, CA
Citizenship: US
Post Office Address: 9229 Regents Rd. L101
La Jolla, CA 92037
United States of America

Full Name of Inventor: BARRY A. MORGAN

Inventor's Signature: BAM Date: 1/29/02
Residence Address: Franklin, Massachusetts
Citizenship: US
Post Office Address: 237 Prospect Street
Franklin, Massachusetts 02038
United States of America

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Page 2 of 2 Pages

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Full Name of Inventor: DEAN SADAT-AALAE

Inventor's Signature: Dean Sadat Aalae

Date: 02, 19, 02

Residence Address: La Jolla, CA

Citizenship: US

Post Office Address: 9229 Regents Rd. L101

La Jolla, CA 92037
United States of America

Full Name of Inventor: BARRY A. MORGAN

Inventor's Signature: _____

Date: _____

Residence Address: Franklin, Massachusetts

Citizenship: US

Post Office Address: 237 Prospect Street

Franklin, Massachusetts 02038
United States of America